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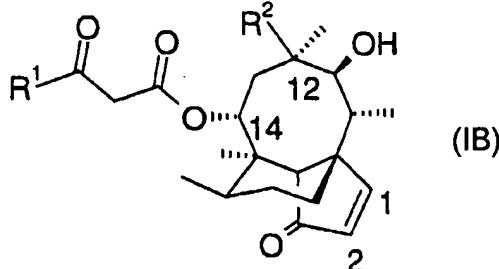
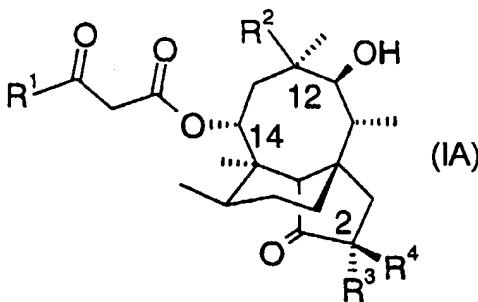
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(54) Title: PLEUROMUTILIN  $\beta$ -KETOESTERS



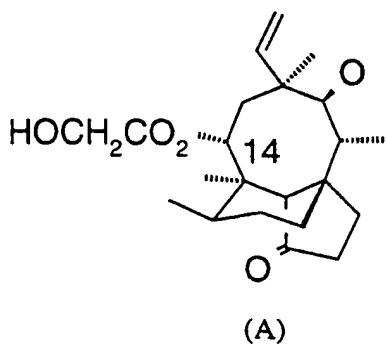
WO 01/14310 A1

(57) Abstract: Pleuromutilin  $\beta$ -ketoesters of Formula (IA) or (IB), in which R<sup>1</sup> is a nitrogen containing heterocycle, an optionally substituted aryl or optionally substituted heteroaryl group, or CH<sub>2</sub>R<sup>5</sup>; R<sup>2</sup> is vinyl or ethyl; R<sup>3</sup> is H, OH or F; and R<sup>4</sup> is H; or R<sup>3</sup> is H and R<sup>4</sup> is F; in which R<sup>5</sup> is halogen or SR<sup>6</sup>; and R<sup>6</sup> is aminoalkyl, a nitrogen containing heterocycle, or an optionally substituted aryl or optionally substituted heteroaryl group, of use in therapy as antibacterial agents.

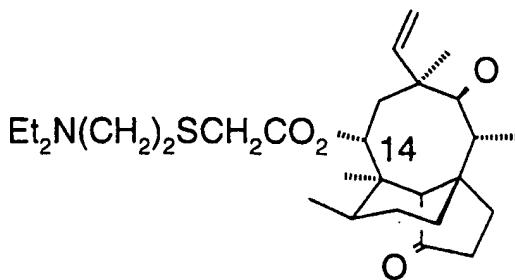
### Pleuromutilin $\beta$ -Ketoesters

The present invention relates to novel compounds, to processes for their preparation, to pharmaceutical compositions containing them and to their use in medical therapy, particularly antibacterial therapy.

Pleuromutilin, the compound of formula (A), is a naturally occurring antibiotic which has anti-mycoplasmal activity and modest antibacterial activity. Mutilin and other compounds with a free OH at C-14 are inactive. The impact of further modification at C-14 on the activity of pleuromutilin has been investigated (H. Egger and H. Reinshagen, *J. Antibiotics*, 1976, 29, 923). Replacing the hydroxy group of the glycolic ester moiety at position 14 by another O, S or N-linked group was found to improve anti-microbial activity. Thus, introducing a diethylaminoethylthio group gives the compound of formula (B), also known as Tiamulin, which is used as a veterinary antibiotic (G. Hogenauer in *Antibiotics*, Vol. V, part 1, ed. F.E. Hahn, Springer-Verlag, 1979, p.344).



(A)



(B)

In this application, the non-conventional numbering system which is generally used in the literature (G. Hogenauer, *loc. cit.*) is used.

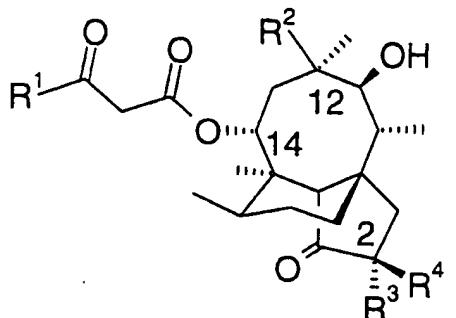
WO 97/25309 (SmithKline Beecham) describes further modification of the acyloxy group, disclosing 14-O-carbamoyl derivatives of mutilin or 19,20-dihydromutilin, in which the N-atom of the carbamoyl group is unsubstituted, mono- or di-substituted.

WO98/05659 (SmithKline Beecham) discloses 14-*O*-carbamoyl derivatives of mutilin or 19,20-dihydromutilin, in which the N-atom of the carbamoyl group is acylated by a group which includes an azabicyclic moiety.

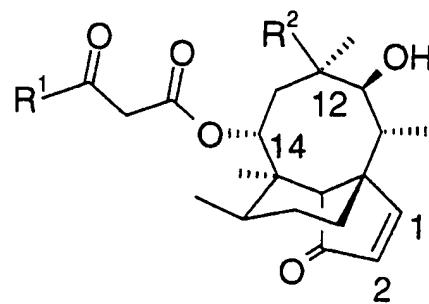
5 WO 99/21855, WO 00/27790 and WO 00/37074 (SmithKline Beecham) describe new classes of mutilin or 19,20-dihydromutilin, in which the glycolic ester moiety at position 14 is further modified.

10 The present invention is based on the unexpected discovery that novel mutilin derivatives having a  $\beta$ -ketoester substituent at the 14-position also have potent antimicrobial activity.

Accordingly the present invention provides a compound of formula (IA) or (IB):



15 (IA)



(IB)

in which:

$R^1$  is a nitrogen containing heterocycle, an optionally substituted aryl or optionally substituted heteroaryl group, or  $CH_2R^5$ ;

$R^2$  is vinyl or ethyl;

20  $R^3$  is H, OH or F; and  $R^4$  is H; or  $R^3$  is H and  $R^4$  is F;

in which:

$R^5$  is halogen or  $SR^6$ ; and

$R^6$  is aminoalkyl, a nitrogen containing heterocycle, or an optionally substituted aryl or optionally substituted heteroaryl group.

Representative examples of R<sup>1</sup> or R<sup>6</sup> when an aryl group include phenyl.

When R<sup>1</sup> or R<sup>6</sup> is aryl, a preferred number of substituents is up to three, more preferred is one. Representative substituents include C<sub>(1-6)</sub>alkoxy, for example methoxy.

5

Representative examples of heteroaryl groups for R<sup>1</sup> or R<sup>6</sup> include 3-pyridyl, 4-pyridyl, pyrimidin-2-yl, 1,3,4-thiadiazol-2-yl, benzothiazol-2-yl, and 2H-1,2,4-triazol-3-yl.

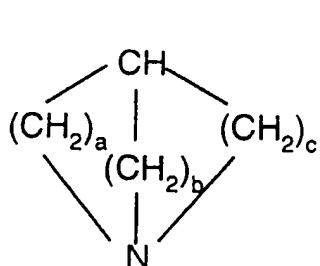
Representative examples of R<sup>1</sup> or R<sup>6</sup> include when an optionally substituted heteroaryl group include 3-pyridyl, 4-pyridyl, pyrimidin-2-yl, 5-amino-1,3,4-thiadiazol-2-yl, 6-

10 ethoxybenzothiazol-2-yl, and 5-amino-2H-1,2,4-triazol-3-yl.

When used herein the term "nitrogen containing heterocycle" refers to a saturated or partially saturated non-aromatic mono- or bicyclic group linked via a ring carbon atom.

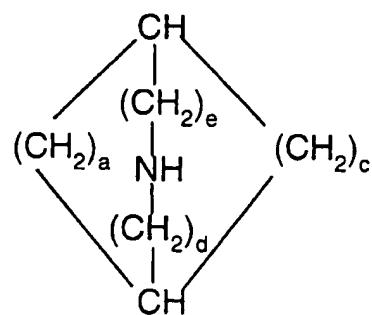
The group may comprise one to three nitrogen atoms, preferably one or two, more

15 preferably one nitrogen atom. When the group is monocyclic it can contain between 4 and 8 ring atoms, and when bicyclic it can contain between 5 and 10 ring atoms in each ring. The azabicyclic ring system may, for example, be represented by the formulae (X) or (Y):



20

(X)



(Y)

wherein each of a, b, c, d and e, which may be the same or different, is for a, b and c an integer from 1 to 4, and for d and e 0 or an integer from 1 to 3, such that any one ring has between 5 and 10 ring atoms.

The nitrogen containing heterocycle can be optionally substituted on carbon by up to 3 substituents. Suitable substituents include C<sub>(1-6)</sub>alkyl, C<sub>(1-6)</sub>alkyloxy, C<sub>(2-6)</sub>alkenyl and C<sub>(2-6)</sub>alkenyloxy, each of which may be carried by either a bridgehead or a non-bridgehead carbon atom. In addition, the or each nitrogen atom may be substituted by

5 oxygen, to form an N-oxide, or by mono- or di-C<sub>(1-6)</sub>alkyl, in which case it will be appreciated that a quaternary cation can be formed. Representative nitrogen substituents include C<sub>(1-6)</sub>alkyl, preferably methyl. The counterion may be a halide ion such as chloride or bromide, preferably chloride. The ring system additionally may contain one or more double bonds.

10

Representative nitrogen containing heterocycles include optionally substituted azabicycloheptyl, azabicyclo-octyl and piperidinyl. Preferred nitrogen containing heterocyclic moieties include optionally substituted 1-aza-bicyclo[2.2.2]oct-4-yl, 1-aza-bicyclo[2.2.1]hept-4-yl, piperidin-4-yl. The linking ring carbon in an azabicyclic system  
15 may be a bridgehead atom or a non-bridgehead atom.

When R<sup>5</sup> is halogen, a preferred value is chlorine.

When R<sup>5</sup> is SR<sup>6</sup>, representative values for R<sup>6</sup> include dialkylaminoalkyl, aza-

20 bicyclo[2.2.2]octyl, pyrimidinyl, aryl, amino-1,3,4-thiadiazolyl, alkoxybenzothiazolyl, and amino-2H-1,2,4-triazolyl. Preferred values for R<sup>6</sup> include 2-diethylaminoethyl, 1-aza-bicyclo[2.2.2]oct-4-yl, pyrimidin-2-yl, phenyl, 5-amino-1,3,4-thiadiazol-2-yl, 6-ethoxybenzothiazol-2-yl, and 5-amino-2H-1,2,4-triazol-3-yl.

25 When used herein the term "aminoalkyl" refers to, unless otherwise defined, a mono- or di-C<sub>(1-6)</sub>alkylamino-C<sub>(1-6)</sub>alkyl group. Representative amino alkyl groups include di-C<sub>(1-6)</sub>alkylamino-C<sub>(1-6)</sub>alkyl, preferred aminoalkyl groups include 2-diethylaminoethyl.

When used herein, the term "aryl" refers to, unless otherwise defined, phenyl or naphthyl

30 optionally substituted with up to five, preferably up to three substituents.

Suitable substituents for an aryl group include, for example, and unless otherwise defined, halogen, (C<sub>1-6</sub>)alkyl, aryl, aryl(C<sub>1-6</sub>)alkyl, (C<sub>1-6</sub>)alkoxy, (C<sub>1-6</sub>)alkoxy(C<sub>1-6</sub>)alkyl, halo(C<sub>1-6</sub>)alkyl, aryl(C<sub>1-6</sub>)alkoxy, hydroxy, nitro, cyano,  
5 azido, amino, mono- and di-N-(C<sub>1-6</sub>)alkylamino, acylamino, arylcarbonylamino, acyloxy, carboxy, carboxy salts, carboxy esters, carbamoyl, mono- and di-N-(C<sub>1-6</sub>)alkylcarbamoyl, (C<sub>1-6</sub>)alkoxycarbonyl, aryloxycarbonyl, ureido, guanidino, (C<sub>1-6</sub>)alkylguanidino, amidino, (C<sub>1-6</sub>)alkylamidino, sulphonylamino, aminosulphonyl, (C<sub>1-6</sub>)alkylthio, (C<sub>1-6</sub>)alkylsulphanyl, (C<sub>1-6</sub>)alkylsulphonyl, heterocyclyl, heteroaryl,  
10 heterocyclyl(C<sub>1-6</sub>)alkyl and heteroaryl(C<sub>1-6</sub>)alkyl. In addition, two adjacent ring carbon atoms may be linked by a (C<sub>3-5</sub>)alkylene chain, to form a carbocyclic ring.

When used herein, the terms "alkyl" and "alkenyl" refer to (individually or as part of alkoxy or alkenyloxy) straight and branched groups containing up to six carbon atoms  
15 and are optionally substituted by one or more groups selected from the group consisting of aryl, heteroaryl, heterocyclyl, (C<sub>1-6</sub>)alkoxy, (C<sub>1-6</sub>)alkylthio, aryl(C<sub>1-6</sub>)alkoxy, aryl(C<sub>1-6</sub>)alkylthio, amino, mono- or di-(C<sub>1-6</sub>)alkylamino, cycloalkyl, cycloalkenyl, carboxy and esters thereof, amide, ureido, guanidino, (C<sub>1-6</sub>)alkylguanidino, amidino, (C<sub>1-6</sub>)alkylamidino, (C<sub>1-6</sub>)acyloxy, azido, hydroxy, and halogen.

20 When used herein, the terms "cycloalkyl" and "cycloalkenyl" refer to groups having from three to eight ring carbon atoms and are optionally substituted as described hereinabove for alkyl and alkenyl groups.

25 When used herein the terms "heterocyclyl" and "heterocyclic" refer to, unless otherwise defined, non-aromatic, single and fused, rings suitably containing up to four heteroatoms in each ring, each of which is selected from oxygen, nitrogen and sulphur, which rings, may be unsubstituted or substituted by, for example, up to three substituents. Each heterocyclic ring preferably has from 4 to 7, preferably 5 or 6, ring atoms. A fused

heterocyclic ring system may include carbocyclic rings and need include only one heterocyclic ring.

Preferably a substituent for a heterocyclyl group is selected from oxo, and the group  
5 hereinbefore defined as suitable aryl substituents.

When used herein, the term "heteroaryl" suitably includes, unless otherwise defined, a mono- or bicyclic heteroaromatic ring system comprising up to four, preferably 1 or 2, heteroatoms each selected from oxygen, nitrogen and sulphur. Each ring may have from  
10 4 to 7, preferably 5 or 6, ring atoms. A bicyclic heteroaromatic ring system may include a carbocyclic ring. Preferably a substituent for a heteroaromatic ring system is selected from the group hereinbefore defined as suitable aryl substituents.

Depending on the substituents, two or more diastereoisomers may be possible. In that  
15 situation the present invention includes the individual diastereoisomers and mixtures thereof.

The 2-hydroxy-substituted compounds of formula (IA) are of the (2S) configuration. The 2-F-substituted compounds of formula (IA) may of (2S) configuration or (2R)  
20 configuration, or be provided as mixtures thereof. The (2S) configuration is however preferred.

Preferred compounds of the invention include:

Mutilin 14-(3-oxo-3-pyridin-3-yl-propionate);  
Mutilin 14-(3-oxo-3-pyridin-4-yl-propionate);  
25 Mutilin 14-[3-(1-benzyl-piperidin-4-yl)-3-oxo-propionate];  
Mutilin 14-[3-(1-aza-bicyclo[2.2.1]hept-4-yl)-3-oxo-propionate];  
Mutilin 14-[3-(1-aza-bicyclo[2.2.2]oct-4-yl)-3-oxo-propionate]; and  
Mutilin 14-[3-(4-methoxyphenyl)-3-oxo-propionate].

30

The compounds of this invention may be in crystalline or non-crystalline form, and, if crystalline, may optionally be hydrated or solvated. This invention includes within its

scope stoichiometric hydrates as well as compounds containing variable amounts of water.

The compounds according to the invention are suitably provided in substantially pure  
5 form, for example at least 50% pure, suitable at least 60% pure, advantageously at least 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight.

Compounds of the invention that contain a basic group such as an amino substituent may  
10 be in the form of a free base or an acid addition salt. Compounds having an acidic group such as a carboxy substituent may be in the form of a pharmaceutically acceptable salt. Compounds of the invention having both a basic and an acidic centre may be in the form of zwitterions, acid addition salt of the basic centre or alkali metal salts (of the carboxy group). Pharmaceutically acceptable salts are preferred. The present invention includes  
15 all such salts.

Pharmaceutically acceptable acid-addition salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.*, 1977, 66, 1-19. Suitable salts include the hydrochloride, maleate, and methanesulphonate; particularly the hydrochloride.

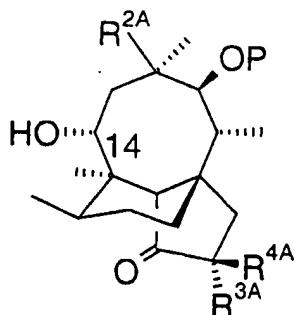
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Pharmaceutically acceptable salts include those described by Berge, Bighley, and Monkhouse, *J. Pharm. Sci.*, 1977, 66, 1-19. Suitable salts include alkali metal salts such as the sodium and potassium salts.

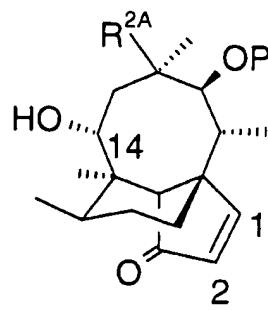
25 Compounds of the present invention may be readily prepared from a pleuromutilin or a 19,20-dihydro-pleuromutilin derivative by adapting procedures well known in the art for forming either ester or  $\beta$ -ketoester groups. Suitable procedures are reviewed in, for example, I.O. Sutherland in *Comprehensive Organic Chemistry*, Vol. 2, ed. I.O. Sutherland, p. 869, Pergamon, 1979; and J.M. Brown, *ibid.*, p. 779.

30

Accordingly, the present invention provides a process for preparing a compound of formula (IA) or (IB) which comprises reacting a compound of formula (IIA) or (IIB):



5 (IIA)



(IIB)

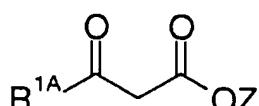
in which:

P is hydrogen or an hydroxy-protecting group;

R<sup>2A</sup>, R<sup>3A</sup> and R<sup>4A</sup> are R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> as defined for formulae (IA) and (IB) or a group convertible to R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> respectively; and

10 is as hereinbefore defined;

with a compound of formula (III):



(III)

in which:

15 R<sup>1A</sup> is R<sup>1</sup> as defined for formulae (IA) and (IB) or a group convertible to R<sup>1</sup>; and Z is C<sub>(1-6)</sub>alkyl;

in a trans-esterification reaction and thereafter, and if so needed;

converting P to hydrogen, and if necessary

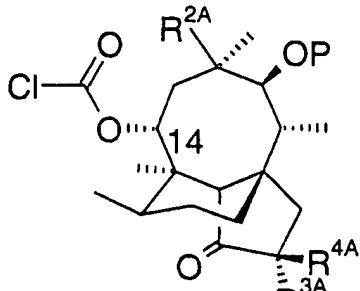
converting an R<sup>2A</sup>, R<sup>3A</sup> or R<sup>4A</sup> group to an R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group.

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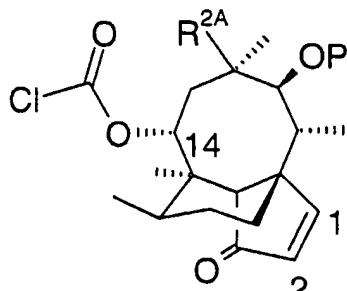
Suitable trans-esterification conditions are well known in the art (e.g. I.O. Sutherland in *Comprehensive Organic Chemistry*, Vol. 2, ed. I.O. Sutherland, p. 869, Pergamon, 1979 and J. Mulzer in *Comprehensive Organic Functional Group Transformation*, Vol. 5, ed. C.J. Moody, p.121, Elsevier Scientific, Oxford, 1995.) and include an organic solvent

such as toluene at a temperature of 20°C to 150°C (preferably 80°C to 150°C), in the presence of a catalyst (acid or base) such as 4-dimethylaminopyridine.

In a further aspect, the present invention provides a process for preparing a compound of  
5 formula (IA) or (IB) which comprises reacting a compound of formula (IVA) or (IVB):



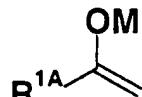
(IVA)



(IVB)

in which P,  $\text{R}^{2A}$ ,  $\text{R}^{3A}$  and  $\text{R}^{4A}$  are as hereinbefore defined;

10 with a compound of formula (V):



(V)

in which  $\text{R}^{1A}$  is as hereinbefore defined, and M is  $\text{Si}(\text{R}^7)_3$ , magnesium or an alkali metal, in which each  $\text{R}^7$  is independently selected from C(1-6)alkyl and phenyl;

15 in a  $\beta$ -ketoester formation reaction and thereafter, and if so needed; converting P to hydrogen, and if necessary, converting an  $\text{R}^{2A}$ ,  $\text{R}^{3A}$  or  $\text{R}^{4A}$  group to an  $\text{R}^2$ ,  $\text{R}^3$  or  $\text{R}^4$  group.

M is preferably lithium.

20

Suitable  $\beta$ -ketoester formation conditions are well known in the art (e.g. J.M. Brown in *Comprehensive Organic Chemistry*, Vol. 2, ed. I.O. Sutherland, p. 779, Pergamon, 1979)

and include an organic solvent such as tetrahydrofuran, at a temperature of -100°C to 0°C (preferably -80°C to -60°C).

In a further aspect this invention also provides for the preparation of compounds of

5 formula (IA) or (IB) in which R<sup>1</sup> is -CH<sub>2</sub>SR<sup>6</sup> and R<sup>1</sup> and R<sup>6</sup> are as hereinbefore defined; which comprises reacting a compound of formula (IA) or (IB); in which R<sup>1</sup> is -CH<sub>2</sub>-halogen with a compound of formula (VI):

10

HSR<sup>6A</sup>

(VI)

in which R<sup>6A</sup> is R<sup>6</sup> as defined for formula (IA) and (IB), or a group convertible to R<sup>6</sup>; and thereafter, and if so needed; converting R<sup>6A</sup> to R<sup>6</sup>.

15

Conversion of an R<sup>1A</sup>, R<sup>2A</sup>, R<sup>3A</sup> or R<sup>4A</sup> group to an R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group typically arises if a protecting group is needed during the above reactions or during the preparation of the reactants by the procedures described below.

20 When P is a hydroxyl protecting group, a preferred protecting group is acyl, for example so that -OP is trifluoroacetoxy or dichloroacetoxy. When the intended R<sup>3</sup> is also hydroxyl, then R<sup>3A</sup> is also preferably acyloxy, for example acetyl or dichloroacetyl. Hydroxyl groups at positions 11 and 2 (as groups OP and R<sup>3A</sup>) may be protected using, for example, trifluoroacetic anhydride or dichloroacetic anhydride and pyridine in

25 tetrahydrofuran or N-trifluoroacetyl-imidazole in tetrahydrofuran at 0°C. After the reaction described above with (III) is complete, the protecting acyl groups may be removed to restore the hydroxyl groups, for instance by hydrolysis e.g. using NaOH in either MeOH or tetrahydrofuran/water solution.

Suitable hydroxy, carboxy and amino protecting groups are those well known in the art and which may be removed under conventional conditions and without disrupting the remainder of the molecule. A comprehensive discussion of the ways in which hydroxy, carboxy and amino groups may be protected and methods for cleaving the resulting

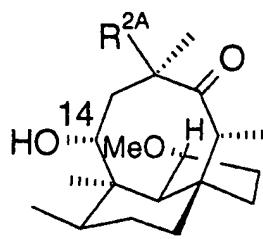
5 protected derivatives is given in for example *Protective Groups in Organic Chemistry*, T.W. Greene and P.G.M. Wuts, (Wiley-Interscience, New York, 2nd edition, 1991). Particularly suitable hydroxy protecting groups include, for example, triorganosilyl groups such as, for instance, trialkylsilyl and also organocarbonyl and organooxycarbonyl groups such as, for instance, acetyl, allyloxycarbonyl and 4-methoxybenzyloxycarbonyl

10 Particularly suitable carboxy protecting groups include alkyl and aryl esters, for instance methyl, ethyl and phenyl. Particularly suitable amino protecting groups include alkoxy carbonyl and 4-methoxybenzyloxycarbonyl.

$R^2A$  is typically the  $R^2$  group vinyl, and this may be converted to the alternative  $R^2$  ethyl group by hydrogenating the vinyl group to form an ethyl group, typically by hydrogenation over a palladium catalyst (e.g. 10% palladium-on-carbon) in a solvent such as ethyl acetate, ethanol, dioxane, or tetrahydrofuran.

$R^3A$  is typically hydrogen, fluoro or protected hydroxyl, such as acyloxy. After the coupling reaction, if required, protecting acyl groups may be removed to restore the hydroxyl groups by hydrolysis e.g. using NaOH in MeOH.

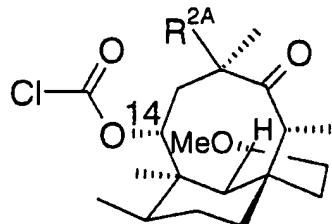
A compound of formula (IA) may also be prepared from an *epi*-mutilin starting material. Accordingly, in a further aspect, the present invention provides a process for preparing a compound of formula (IA) in which  $R^3$  and  $R^4$  are both hydrogen which comprises reacting an *epi*-mutilin compound of formula (IIC):



(IIC)

wherein  $R^{2A}$  is as hereinbefore defined;  
with a compound (III), as hereinbefore defined;  
5 and then treating the product with an acid;  
and where required or desired converting an  $R^{1A}$  group to an  $R^1$  group and an  $R^{2A}$  group to an  $R^2$  group.

In a yet further aspect, the present invention provides a process for preparing a compound  
10 of formula (IA) in which  $R^3$  and  $R^4$  are both hydrogen which comprises reacting an *epi*-mutilin compound of formula (IVC):



(IVC)

15 wherein  $R^{2A}$  is as hereinbefore defined;  
with a compound (V), as hereinbefore defined;  
and then treating the product with an acid;  
and where required or desired converting an  $R^{1A}$  group to an  $R^1$  group and an  $R^{2A}$  group to an  $R^2$  group.

20

The acid treatment indicated above converts the *epi*-mutilin configuration to the usual mutilin nucleus of formula (IIA). Typically this conversion is carried out by treatment with conc. HCl or Lukas reagent (conc. HCl saturated with ZnCl<sub>2</sub>) in dioxane.

- 5 It should be appreciated that it may be necessary to interconvert one R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group to another R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group. This typically arises when one compound of formula (IA/B) is used as the immediate precursor of another compound of formula (IA/B) or when it is easier to introduce a more complex or reactive substituent at the end 10 of a synthetic sequence. A substituent group in R<sup>1</sup> can be converted into another substituent group using one of the general methods for functional group transformation described in the literature (*e.g.* a carboxylic ester can be hydrolysed to a carboxylic acid with base; an acid can be converted into an amide; a *tert*-butoxycarbonylamino group can be converted into an amine by treatment with trifluoroacetic acid; an amino group can be alkylated or acylated), provided that the method chosen is compatible with other 15 functional groups in the molecule (*e.g.* the ketone at C-3 in the pleuromutilin nucleus).

Functional group transformations are well known in the art and are described in, for instance, *Comprehensive Organic Functional Group Transformations*, eds. A.R. Katritzky, O. Meth-Cohn, and C.W. Rees (Elsevier Science Ltd., Oxford, 1995), 20 *Comprehensive Organic Chemistry*, eds. D. Barton and W.D. Ollis (Pergamon Press, Oxford, 1979), and *Comprehensive Organic Transformations*, R.C. Larock (VCH Publishers Inc., New York, 1989).

- Compounds of formulae (IIA) in which R<sup>3A</sup> and R<sup>4A</sup> are hydrogen, (IIB) and (IIC) may 25 be readily prepared according to methods described in the literature, for example G. Schulz and H. Berner, *Tetrahedron*, 1984, 40, 905, and in WO 97/25309 and WO 98/05659 (SmithKline Beecham). Where necessary, and as hereinbefore described, saponification of the C-14 ester may be carried out at an appropriate stage.
- 30 Compounds of formula (IIA) in which R<sup>3A</sup> is hydroxyl or fluoro may be prepared from pleuromutilin, via an intermediate 2-diazo compound, the preparation of which is

described by G. Schulz and H. Berner in *Tetrahedron*, 1984, 40, 905. Where necessary, saponification of the C-14 ester group may be carried out at an appropriate stage using conventional techniques such as sodium hydroxide or sodium methoxide in methanol or aqueous tetrahydrofuran solution.

5

The intermediate 2-diazo compound may be reacted with a carboxylic acid to give a 2-acyloxy-mutilin derivative. Suitably, reaction with dichloroacetic acid gives a 2-dichloroacetoxy-mutilin derivative, which can be deprotected as described above to provide the (2S)-2-hydroxy derivative, at an appropriate stage.

10

Compounds of formula (IIA) in which R<sup>3A</sup> is fluoro may be obtained by reacting 2-diazo-mutilin with a source of hydrogen fluoride. Conveniently, the hydrogen fluoride source is an amine complex of hydrogen fluoride such as hydrogen fluoride-pyridine. The reaction may be carried out in an anhydrous solvent (*e.g.* diethyl ether,

15 tetrahydrofuran, 1,2-dimethoxyethane), at a temperature of -15°C to 25°C. This reaction produces (2S)-2-fluoro derivatives. (2R)-2-Fluoro-mutilin derivatives may be prepared by treating the (2S)-isomer with a base (*e.g.* sodium hydroxide or potassium hydroxide in ethanol). This will usually produce a mixture of (2S) and (2R)-isomers that may be separated using conventional techniques such as chromatography and crystallisation.

20

Compounds of formula (III) are available commercially, or may be readily prepared by adapting procedures well known in the art *e.g.* by reacting a suitable methyl ketone with methyl cyanoformate in a solvent such as tetrahydrofuran in the presence of a suitable base such as lithium diisopropylamide at 0°C to -100°C (preferably -80°C to -60°C)

25 (L.N. Mander *et al*, *Tetrahedron Lett.*, 1983, 24, 5425). Other procedures well known in the art for preparing β-ketoesters can be found in *e.g.* J.M. Brown in *Comprehensive Organic Chemistry*, Vol. 2, ed. I.O. Sutherland, p. 1017, Pergamon, 1979.

30 Enolates of formula (V) may be readily prepared from the corresponding ketone by methods known to those skilled in the art. Ketones may be prepared by adapting

procedures well known in the art for preparing ketones (e.g. A.J. Waring in *Comprehensive Organic Chemistry*, Vol. 1, ed. J.F. Stoddart, p. 1017, Pergamon, 1979).

Compounds of formula (VI) are available commercially, or may be readily prepared by  
5 adapting procedures well known in the art for preparing thiols (e.g. G.C. Barrett in *Comprehensive Organic Chemistry*, Vol. 3, ed. D.N. Jones, p.3, Pergamon, 1979).

The compounds of the present invention may contain a chiral centre, and therefore the above processes may produce a mixture of diastereoisomers. A single diastereoisomer  
10 may be prepared by separating such a mixture of diastereoisomers by conventional techniques such as chromatography or fractional crystallisation.

The compounds of this invention may be in crystalline or non-crystalline form, and, if crystalline, may optionally be hydrated or solvated. When some of the compounds of this  
15 invention are allowed to crystallise or are recrystallised from organic solvents, solvent of crystallisation may be present in the crystalline product. Similarly, some of the compounds of this invention may be crystallised or recrystallised from solvents containing water. In such cases water of hydration may be present in the crystalline product. Crystallisation procedures will usually produce stoichiometric hydrates.  
20 Compounds containing variable amounts of water may be produced by processes such as lyophilisation.

The compounds according to the invention are suitably provided in substantially pure form, for example at least 50% pure, suitable at least 60% pure, advantageously at least  
25 75% pure, preferably at least 85% pure, more preferably at least 95% pure, especially at least 98% pure, all percentages being calculated as weight/weight. An impure or less pure form of a compound according to the invention may, for example, be used in the preparation of a more pure form of the same compound or of a related compound (for example a corresponding derivative) suitable for pharmaceutical use.

30 The present invention also includes pharmaceutically acceptable salts and derivatives of the compounds of the invention. Salt formation may be possible when one of the

substituents carries an acidic or basic group. Salts may be prepared by salt exchange in conventional manner.

5 Acid-addition salts may be pharmaceutically acceptable or non-pharmaceutically acceptable. In the latter case, such salts may be useful for isolation and purification of the compound of the invention, or intermediates thereto, and will subsequently be converted into a pharmaceutically acceptable salt or the free base.

10 The compounds of the present invention and their pharmaceutically acceptable salts or derivatives have antimicrobial properties and are therefore of use in therapy, in particular for treating microbial infections in animals, especially mammals, including humans, in particular humans and domesticated animals (including farm animals). The compounds may be used for the treatment of infections caused by, for example, Gram-positive and Gram-negative bacteria and mycoplasmas, including, for example, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, *Haemophilus sp.*, *Neisseria sp.*, *Legionella sp.*, *Chlamydia sp.*, *Moraxella catarrhalis*, *Mycoplasma pneumoniae*, and *Mycoplasma gallisepticum*.

20 The present invention also provides a method of treating microbial infections in animals, especially in humans and in domesticated mammals, which comprises administering a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof, or a composition according to the invention, to a patient in need thereof.

25 The invention further provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament for use in the treatment of microbial infections.

30 Compounds of the present invention may be used to treat skin and soft tissue infections and acne, by topical application. Accordingly, in a further aspect the present invention provides the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the preparation of a medicament adapted for topical

administration for use in the treatment of skin and soft tissue infections and also in the treatment of acne in humans.

Compounds of the present invention may be also used for the elimination or reduction of 5 nasal carriage of pathogenic bacteria such as *S. aureus*, *H. influenzae*, *S. pneumonia* and *M. catarrhalis*, in particular colonisation of the nasopharynx by such organisms, by the administration of a compound of the present invention thereto. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a 10 medicament adapted for administration to the nasal cavity, for reducing or eliminating the nasal carriage of pathogenic organisms. Preferably, the medicament is adapted for focussed delivery to the nasopharynx, in particular the anterior nasopharynx.

Such reduction or elimination of nasal carriage is believed to be useful in prophylaxis of 15 recurrent acute bacterial sinusitis or recurrent otitis media in humans, in particular in reducing the number of episodes experienced by a patient over a given period of time or increasing the time intervals between episodes. Accordingly, in a further aspect, the present invention provides for the use of a compound of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a 20 medicament adapted for administration to the nasal cavity, for prophylaxis of recurrent acute bacterial sinusitis or recurrent otitis media.

Compounds of the present invention are also useful in treating chronic sinusitis. Accordingly, in a further aspect, the present invention provides for the use of a compound 25 of the invention or a pharmaceutically acceptable salt or derivative or solvate thereof in the manufacture of a medicament, for treating of chronic sinusitis.

The compounds according to the invention may suitably be administered to the patient at a daily dosage of from 1.0 to 50 mg/kg of body weight. For an adult human (of 30 approximately 70 kg body weight), from 50 to 3000 mg, for example about 1500 mg, of a compound according to the invention may be administered daily. Suitably, the dosage for

adult humans is from 5 to 20 mg/kg per day. Higher or lower dosages may, however, be used in accordance with normal clinical practice.

To lessen the risk of encouraging the development of resistant organisms during

5 prophylaxis of recurrent otitis media or recurrent acute bacterial sinusitis, it is preferred to administer the drug on an intermittent, rather than a continual, basis. In a suitable intermittent treatment regimen for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is administered on a daily basis, for a small number of days, for instance from 2 to 10, suitably 3 to 8, more suitably about 5 days, the administration then  
10 being repeated after an interval, for instance, on a monthly basis over a period of months, for instance up to six months. Less preferably, the drug substance may be administered on a continuing, daily basis, over a prolonged period, for instance several months.  
Suitably, for prophylaxis of recurrent otitis media or recurrent sinusitis, drug substance is  
15 administered once or twice a day. Suitably, drug substance is administered during the winter months when bacterial infections such as recurrent otitis media and recurrent sinusitis tend to be more prevalent. The drug substance may be administered at a dosage of from 0.05 to 1.00mg, typically about 0.1 to 0.2mg, in each nostril, once or twice a day.

More generally, the compounds and compositions according to the invention may be

20 formulated for administration in any convenient way for use in human or veterinary medicine, by analogy with other antibiotics.

Accordingly, in a further aspect, the present invention provides a pharmaceutical

composition comprising a compound of the invention or a pharmaceutically acceptable

25 salt or derivative or solvate thereof together with a pharmaceutically acceptable carrier or excipient.

The compounds and compositions according to the invention may be formulated for

administration by any route, for example oral, topical or parenteral. The compositions

30 may, for example, be made up in the form of tablets, capsules, powders, granules, lozenges, creams, syrups, sprays or liquid preparations, for example solutions or

suspensions, which may be formulated for oral use or in sterile form for parenteral administration by injection or infusion.

Tablets and capsules for oral administration may be in unit dosage form, and may contain  
5 conventional excipients including, for example, binding agents, for example, syrup, acacia, gelatin, sorbitol, tragacanth, or polyvinylpyrrolidone; fillers, for example lactose, sugar, maize-starch, calcium phosphate, sorbitol or glycine; tabletting lubricants, for example magnesium stearate, talc, polyethylene glycol or silica; disintegrants, for example potato starch; and pharmaceutically acceptable wetting agents, for example  
10 sodium lauryl sulphate. The tablets may be coated according to methods well known in normal pharmaceutical practice.

Oral liquid preparations may be in the form of, for example, aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for  
15 reconstitution with water or another suitable vehicle before use. Such liquid preparations may contain conventional additives, including, for example, suspending agents, for example sorbitol, methyl cellulose, glucose syrup, gelatin, hydroxyethyl cellulose, carboxymethyl cellulose, aluminium stearate gel or hydrogenated edible fats; emulsifying agents, for example lecithin, sorbitan monooleate or acacia; non-aqueous vehicles (which  
20 may include edible oils), for example almond oil, oily esters (for example glycerine), propylene glycol, or ethyl alcohol; preservatives, for example methyl or propyl p-hydroxybenzoate or sorbic acid; and, if desired, conventional flavouring and colour agents.

25 Compositions according to the invention intended for topical administration may, for example, be in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, nose drops, nasal sprays, impregnated dressings, and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical  
30 formulations may also contain compatible conventional carriers, for example cream or ointment bases, ethanol or oleyl alcohol for lotions and aqueous bases for sprays. Such

carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

Compositions according to the invention intended for topical administration, in addition  
5 to the above, may also contain a steroid anti-inflammatory agent; for example,  
betamethasone.

Compositions according to the invention may be formulated as suppositories, which may contain conventional suppository bases, for example cocoa-butter or other glycerides.

10

Compositions according to the invention intended for parenteral administration may conveniently be in fluid unit dosage forms, which may be prepared utilizing the compound and a sterile vehicle, water being preferred. The compound, depending on the vehicle and concentration used, may be either suspended or dissolved in the vehicle. In  
15 preparing solutions, the compound may be dissolved in water for injection and filter-sterilised before being filled into a suitable vial or ampoule, which is then sealed. Advantageously, conventional additives including, for example, local anaesthetics, preservatives, and buffering agents can be dissolved in the vehicle. In order to enhance the stability of the solution, the composition may be frozen after being filled into the vial,  
20 and the water removed under vacuum; the resulting dry lyophilised powder may then be sealed in the vial and a accompanying vial of water for injection may be supplied to reconstitute the liquid prior to use. Parenteral suspensions may be prepared in substantially the same manner except that the compound is suspended in the vehicle instead of being dissolved and sterilisation cannot be accomplished by filtration. The  
25 compound may instead be sterilised by exposure to ethylene oxide before being suspended in the sterile vehicle. Advantageously, a surfactant or wetting agent is included in such suspensions in order to facilitate uniform distribution of the compound.

A compound or composition according to the invention is suitably administered to the  
30 patient in an anti-microbially effective amount.

A composition according to the invention may suitably contain from 0.001% by weight, preferably (for other than spray compositions) from 10 to 60% by weight, of a compound according to the invention (based on the total weight of the composition), depending on the method of administration.

5

When the compositions according to the invention are presented in unit dosage form, for instance as a tablet, each unit dose may suitably comprise from 25 to 1000 mg, preferable from 50 to 500 mg, of a compound according to the invention.

- 10 Representative compositions of the present invention include those adapted for intranasal administration, in particular, those that will reach into the nasopharynx. Such compositions are preferably adapted for focussed delivery to, and residence within, the nasopharynx. The term 'focussed delivery' is used to mean that the composition is delivered to the nasopharynx, rather than remaining within the nares. The term
- 15 'residence' within the nasopharynx is used to mean that the composition, once delivered to the nasopharynx, remains within the nasopharynx over a course of several hours, rather than being washed away more or less immediately. Preferred compositions include spray compositions and creams. Representative spray compositions include aqueous compositions, as well as oily compositions that contain amphiphilic agents so that the
- 20 composition increases in viscosity when in contact with moisture. Creams may also be used, especially creams having a rheology that allows the cream to spread readily in the nasopharynx.

Preferred aqueous spray compositions include, in addition to water, further excipients

- 25 including a tonicity modifier such as a salt, for instance sodium chloride; preservative, such as benzalkonium salt; a surfactant such as a non-ionic surfactant, for instance a polysorbate; and buffer, such as sodium dihydrogen phosphate; present in low levels, typically less than 1%. The pH of the composition may also be adjusted, for optimum stability of the drug substance during storage. For compounds of the present invention, a
- 30 pH in the range 5 to 6, preferably about 5.3 to 5.8, typically about 5.5 is optimal

Representative oily spray and cream compositions are described in WO 98/14189 (SmithKline Beecham). Representative aqueous sprays are described in International Application no PCT/GB98/03211 (SmithKline Beecham).

- 5     Suitably, the drug substance is present in compositions for nasal delivery in between 0.001 and 5%, preferably 0.005 and 3%, by weight of the composition. Suitable amounts include 0.5% and 1% by weight of the composition (for oily compositions and creams) and from 0.01 to 0.2% (aqueous compositions).
- 10    Spray compositions according to the present invention may be delivered to the nasal cavity by spray devices well known in the art for nasal sprays, for instance an air lift pump. Preferred devices include those that are metered to provide a unit volume of composition, preferably about 100 $\mu$ l, and optionally adapted for nasal administration by addition of a modified nozzle.
- 15

The invention is illustrated by the following Examples.

**Example 1. Mutilin 14-(3-oxo-3-phenyl-propionate)**

20    **Step 1. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-(3-oxo-3-phenyl-propionate)**

A solution of (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin {H. Berner *et al*, Tetrahedron, 1980, 36, 1807} (1.0 g, 3.0 mmol) in toluene (15 ml) was treated with ethyl 3-oxo-3-phenyl-propionate (0.523 ml, 3.0 mmol) and 4-dimethylaminopyridine (183 mg, 1.5 mmol). The reaction mixture was heated to reflux for 16 hours and then diluted with 25 ethyl acetate and washed with dilute hydrochloric acid. After drying ( $MgSO_4$ ) purification was accomplished by chromatography on silica gel eluting with 1% ethyl acetate in dichloromethane. The title compound was isolated as a colourless foam (1.03 g, 72%); MS(Electrospray) *m/z* 479 [M-H]<sup>-</sup>.

**Step 2. Mutilin-14-(3-oxo-3-phenyl-propionate)**

30    The product of Step 1 (280 mg, 0.58 mmol) in dioxane (5 ml) was treated with concentrated HCl (5 ml) and the reaction stirred at room temperature for 4 hours. The solution was poured into ethyl acetate and saturated aqueous sodium hydrogen carbonate

solution. The aqueous phase was re-extracted with ethyl acetate and the combined organic phases were washed with brine. The organic phase was dried ( $\text{MgSO}_4$ ) and purified by chromatography on silica gel eluting with 5% ethyl acetate in dichloromethane. The title compound was isolated as a colourless foam (154 mg, 57%);

5 MS(Electrospray)  $m/z$  465 [M-H]<sup>-</sup>.

The following examples were prepared by the general two-step method described in Example 1.

	<b>Example</b>	<b>Beta-ketoester</b>	<b>Characterisation</b>
<b>2</b>	Mutilin 14-(3-oxo-3-pyridin-3-yl-propionate) hydrochloride	Ethyl 3-oxo-3-pyridin-3-yl-propionate	$m/z$ 468 [M+H] <sup>+</sup> .
<b>3</b>	Mutilin 14-(3-oxo-3-pyridin-4-yl-propionate)	Ethyl 3-oxo-3-pyridin-4-yl-propionate	$m/z$ 468 [M+H] <sup>+</sup> .
<b>4</b>	Mutilin 14-[3-(1-benzyl-piperidin-4-yl)-3-oxo-propionate] hydrochloride	Ethyl 3-(1-benzyl-piperidin-4-yl)-3-oxo-propionate <i>Reagent 1</i>	$m/z$ 564 [M+H] <sup>+</sup> .
<b>5</b>	Mutilin 14-[3-(1-aza-bicyclo[2.2.1]hept-4-yl)-3-oxo-propionate] hydrochloride	Methyl 3-(1-aza-bicyclo[2.2.1]hept-4-yl)-3-oxo-propionate <i>Reagent 2</i>	$m/z$ 486 [M+H] <sup>+</sup> .
<b>6</b>	Mutilin 14-[3-(1-aza-bicyclo[2.2.2]oct-4-yl)-3-oxo-propionate] hydrochloride	Methyl 3-(1-aza-bicyclo[2.2.2]oct-4-yl)-3-oxo-propionate <i>Reagent 3</i>	$m/z$ 500 [M+H] <sup>+</sup> .
<b>7</b>	Mutilin 14-(4-chloro-3-oxo-butyrate)	Ethyl 4-chloroacetoacetate	

**Example 8. Mutilin 14-[4-(2-Diethylamino-ethylsulfanyl)-3-oxo-butyrate]**

Sodium (35 mg, 1.5 mmol) was dissolved in dry ethanol (5 ml) and stirred for 15 minutes. 2-Diethylaminoethanethiol hydrochloride (80 mg, 0.45 mmol) was added and the suspension stirred for 5 minutes. A solution of the product of Example 7 (200 mg, 0.45 mmol) in ethanol (2 ml) was added and the mixture stirred for 18 hours. The solution was concentrated, the residue partitioned between ethyl acetate-water and acidified with 2M hydrochloric acid. After separation of the layers the pH of the aqueous phase was adjusted to pH8 (saturated aqueous sodium hydrogen carbonate) and extracted with dichloromethane. The organic phase was washed with brine, dried (sodium sulphate) and purified by chromatography on silica gel eluting with 10% (9:1 methanol: 35% ammonia solution) in dichloromethane. The title compound was isolated as a yellow oil (81 mg, 33%); MS(Electrospray)  $m/z$  536 [M+H]<sup>+</sup>.

The following examples were prepared by the general method described in Example 8.

Example		Thiol	Characterisation*
9	Mutilin 14-[4-(1-aza-bicyclo[2.2.2]oct-4-ylsulfanyl)-3-oxo-butyrate]	1-Aza-bicyclo[2.2.2]octane-4-thiol	$m/z$ 546 [M+H] <sup>+</sup>
10	Mutilin 14-[3-oxo-4-(pyrimidin-2-ylsulfanyl)-butyrate]	Pyrimidine-2-thiol	$m/z$ 515 [M+H] <sup>+</sup>
11	Mutilin 14-[3-oxo-4-phenylsulfanyl-butyrate]	Thiophenol	$m/z$ 511 [M-H] <sup>-</sup>
12	Mutilin 14-[4-(5-amino-1,3,4-thiadiazol-2-ylsulfanyl)-3-oxo-butyrate]	5-Amino-1,3,4-thiadiazole-2-thiol	$m/z$ 536 [M+H] <sup>+</sup>
13	Mutilin 14-[4-(6-ethoxybenzothiazol-2-ylsulfanyl)-3-oxo-butyrate]	6-Ethoxy-benzothiazole-2-thiol	$m/z$ 614 [M+H] <sup>+</sup>
14	Mutilin 14-[4-(5-amino-	5-Amino-2H-1,2,4-triazole-3-	$m/z$ 519

	2H-1,2,4-triazol-3-ylsulfanyl)-3-oxo-butyrate]	thiol	[M+H] <sup>+</sup>
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\* MS(Electrospray)

**Example 15. Mutilin 14-[3-(4-methoxy-phenyl)-3-oxo-propionate)**

**Step 1. (3*R*)-3-Deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin 14-[3-(4-methoxy-phenyl)-3-oxo-propionate)**

- 5 A solution of 4'-methoxyacetophenone (189 mg, 1.26 mmol) in THF (2 ml) was added dropwise to a solution of LDA (0.630 ml, 2.0M solution in heptane/tetrahydrofuran/ethylbenzene, 1.26 mmol) in THF (5ml) at -78°C. The reaction mixture was stirred at -78°C for 45 minutes and then a solution of (3*R*)-3-deoxo-11-deoxy-3-methoxy-11-oxo-4-*epi*-mutilin-14-chloroformate {J.D. Hinks, A.K. Takle and E. Hunt WO 97/25309}
- 10 (500mg, 1.26 mmol) in THF (2 ml) was added. The reaction was stirred at -78°C for 5 minutes and then warmed to room temperature and stirred for a further 2.5 hours. The mixture was poured into water and the product extracted into dichloromethane (x2). The organic phase was dried ( $\text{MgSO}_4$ ) and purified by chromatography on silica gel eluting with 5% ethyl acetate in hexane. The title compound was isolated as a colourless foam
- 15 (92 mg, 14%); MS(Electrospray)  $m/z$  509 [M-H]<sup>-</sup>.

**Step 2. Mutilin 14-[3-(4-methoxy-phenyl)-3-oxo-propionate)**

The product of Step 1 (85 mg, 0.17 mmol) in dioxane (2 ml) was treated with conc. HCl (2 ml) and the reaction stirred at room temperature for 4 hours. The solution was poured into ethyl acetate and saturated aqueous sodium hydrogen carbonate solution. The

- 20 aqueous phase was re-extracted with ethyl acetate and the combined organic phases were washed with brine. The organic phase was dried ( $\text{MgSO}_4$ ) and purified by chromatography on silica gel eluting with 40% ethyl acetate in hexane. The title compound was isolated as a colourless foam (63 mg, 74%); MS(Electrospray)  $m/z$  495 [M-H]<sup>-</sup>.

## Reagents

### Reagent 1. Ethyl 3-(1-benzyl-piperidin-4-yl)-3-oxo-propionate

Sodium hydride (3.0 g, 75 mmol, 60% dispersion in oil) was added portionwise to a stirred solution of ethanol (3.45 g, 75 mmol) in toluene (100 ml). The mixture was stirred at 65°C and a mixture of ethyl acetate (8.8 g, 100 mmol) and ethyl 1-benzyl-piperidine-4-carboxylate (12.4 g, 50 mmol) was added dropwise over 30 minutes. The reaction mixture was stirred at 80°C for 72 hours. After cooling the solution was diluted with ethyl acetate and neutralised with aqueous acetic acid. The organic solution was washed with saturated sodium chloride solution, dried ( $\text{MgSO}_4$ ) and evaporated. Purification of the product by chromatography on silica gel eluting with 25% ethyl acetate in hexanes gave the title compound as an oil (550 mg, 4%):MS(Electrospray)  $m/z$  290 [M+H]<sup>+</sup>.

### Reagent 2. Methyl 3-(1-aza-bicyclo[2.2.1] hept-4-yl)-3-oxo-propionate

A solution of 4-acetyl-1-azabicyclo[2.2.1]heptane (1.4 g, 10 mmol) in tetrahydrofuran (10 ml) was added dropwise to a solution of LDA (6 ml, 2.0M solution in heptane/tetrahydrofuran/ethylbenzene, 12 mmol) in tetrahydrofuran (5ml) at -78°C. After stirring at -78°C for 15 minutes the solution was stirred at 0°C for 60 minutes and then re-cooled to -78°C. Methylcyanoformate (1.02g, 1.0 ml, 12 mmol) was added and the mixture was stirred at -78°C for 30 minutes and then allowed to warm to 0°C. The mixture was poured into saturated ammonium chloride solution and the product extracted into chloroform. The organic phase was dried ( $\text{MgSO}_4$ ) and evaporated. Purification of the residue by chromatography on silica gel eluting with chloroform-methanol-35% ammonia solution (20:1:0.1 v/v/v) gave the title compound as an off white solid (850 mg, 43%):MS(Electrospray)  $m/z$  198 [M+H]<sup>+</sup>.

### Reagent 3. Methyl 3-(1-aza-bicyclo[2.2.2] oct-4-yl)-3-oxo-propionate

4-Acetyl-1-azabicyclo[2.2.2]octane (850 mg, 5.55 mmol) was treated with LDA (3.3 ml, 2.0M solution in heptane/tetrahydrofuran/ethylbenzene, 6.6 mmol) and methylcyanoformate (0.55 ml, 6.6 mmol) in tetrahydrofuran according to the procedure described for Reagent 2, giving the title compound as a gum (740 mg, 64%): MS(Electrospray)  $m/z$  212 [M+H]<sup>+</sup>.

**Biological Data**

Compounds of the present invention were assessed for anti-bacterial activity in a conventional MIC assay against a range of pathogenic organisms.

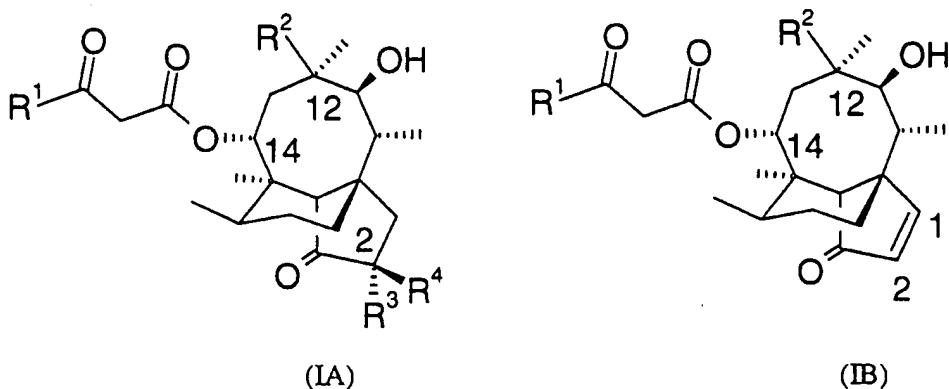
5

Compounds were found to have MICs in the range 0.06 to 4 µg/ml against *Staph aureus* Oxford and 0.06 to 64 µg/ml against *Strep pneumoniae* (R6).

**Claims**

1. A compound of Formula (IA) or (IB):

5



(IA)

(IB)

in which:

R<sup>1</sup> is a nitrogen containing heterocycle, an optionally substituted aryl or optionally substituted heteroaryl group, or CH<sub>2</sub>R<sup>5</sup>;

10 R<sup>2</sup> is vinyl or ethyl;

R<sup>3</sup> is H, OH or F; and R<sup>4</sup> is H; or R<sup>3</sup> is H and R<sup>4</sup> is F;

in which:

R<sup>5</sup> is halogen or SR<sup>6</sup>; and

15 R<sup>6</sup> is aminoalkyl, a nitrogen containing heterocycle, or an optionally substituted aryl or optionally substituted heteroaryl group.

2. A compound as claimed in claim 1 in which R<sup>1</sup> is optionally substituted phenyl, 3-pyridyl, 4-pyridyl, pyrimidin-2-yl, 1, 3, 4-thiadiazol-2-yl, benzothiazol-2-yl, 2H-1, 2, 4-20 triazol-3-yl, azabicycloheptyl, azabicyclo-octyl and piperidinyl.

3. A compound as claimed in claim 1 or 2 in which R<sup>6</sup> is optionally substituted phenyl, 3-pyridyl, 4-pyridyl, pyrimidin-2-yl, 1, 3, 4-thiadiazol-2-yl, benzothiazol-2-yl, 2H-1, 2, 4-triazol-3-yl, azabicycloheptyl, azabicyclo-octyl and piperidinyl

4. A compound as claimed in any one of claims 1 to 3 in which R<sup>5</sup> is SR<sup>6</sup> and R<sup>6</sup> is dialkylaminoalkyl, aza-bicyclo[2.2.2]octyl, pyrimidinyl, aryl, amino-1, 3, 4-thiadiazolyl, alkoxybenzothiazolyl, and amino-2H-1, 2, 4-triazolyl.

5

5. A compound as claimed in claim 1 selected from:

Mutilin 14-(3-oxo-3-phenyl-propionate);

Mutilin 14-(3-oxo-3-pyridin-3-yl-propionate);

Mutilin 14-(3-oxo-3-pyridin-4-yl-propionate);

10 Mutilin 14-[3-(1-benzyl-piperidin-4-yl)-3-oxo-propionate];

Mutilin 14-[3-(1-aza-bicyclo[2.2.1]hept-4-yl)-3-oxo-propionate];

Mutilin 14-[3-(1-aza-bicyclo[2.2.2]oct-4-yl)-3-oxo-propionate];

Mutilin 14-(4-chloro-3-oxo-butyrate);

Mutilin 14-[4-(2-Diethylamino-ethylsulfanyl)-3-oxo-butyrate];

15 Mutilin 14-[4-(1-aza-bicyclo[2.2.2]oct-4-ylsulfanyl)-3-oxo-butyrate];

Mutilin 14-[3-oxo-4-(pyrimidin-2-ylsulfanyl)-butyrate];

Mutilin 14-[3-oxo-4-phenylsulfanyl-butyrate];

Mutilin 14-[4-(5-amino-1,3,4-thiadiazol-2-ylsulfanyl)-3-oxo-butyrate];

Mutilin 14-[4-(6-ethoxybenzothiazol-2-ylsulfanyl)-3-oxo-butyrate];

20 Mutilin 14-[4-(5-amino-2H-1,2,4-triazol-3-ylsulfanyl)-3-oxo-butyrate]; and

Mutilin 14-[3-(4-methoxyphenyl)-3-oxo-propionate].

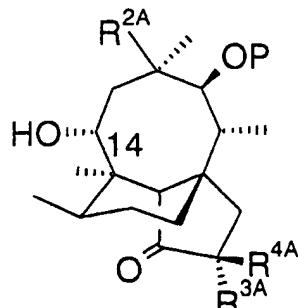
6. A pharmaceutical composition comprising a compound according to claim 1 and a pharmaceutically acceptable carrier.

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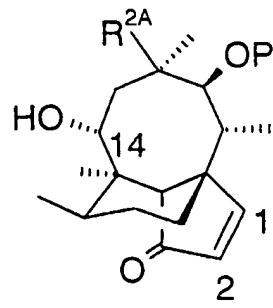
7. A method of treating microbial infections in animals, especially in humans and in domesticated mammals, which comprises administering a compound according to claim 1 to a patient in need thereof.

30 8. A process for preparing a compound of formula (IA) or (IB) as claimed in claim 1 which process comprises

(a) reacting a compound of formula (IIA) or (IIB):



(IIA)



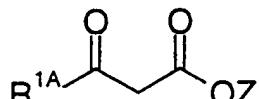
(IIB)

5 in which:

P is hydrogen or an hydroxy-protecting group;

R<sup>2A</sup>, R<sup>3A</sup> and R<sup>4A</sup> are R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> as defined for formulae (IA) and (IB) or a group convertible to R<sup>2</sup>, R<sup>3</sup> and R<sup>4</sup> respectively; and  
is as hereinbefore defined;

10 with a compound of formula (III):



(III)

in which:

R<sup>1A</sup> is R<sup>1</sup> as defined for formulae (IA) and (IB) or a group convertible to R<sup>1</sup>; and

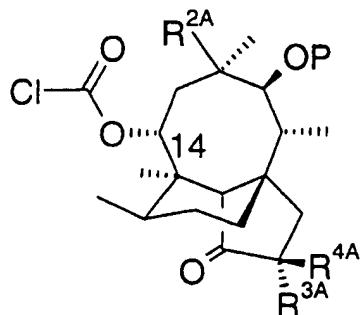
15 Z is C<sub>(1-6)</sub>alkyl;

in a trans-esterification reaction and thereafter, and if so needed;

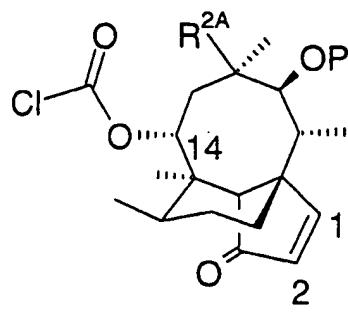
converting P to hydrogen, and if necessary

converting an R<sup>2A</sup>, R<sup>3A</sup> or R<sup>4A</sup> group to an R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group;

20 (b) reacting a compound of formula (IVA) or (IVB):



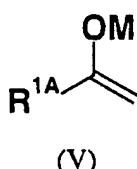
(IV A)



(IV B)

in which P, R<sup>2A</sup>, R<sup>3A</sup> and R<sup>4A</sup> are as hereinbefore defined;

with a compound of formula (V):



(V)

in which R<sup>1A</sup> is as hereinbefore defined, and M is Si(R<sup>7</sup>)<sub>3</sub>, magnesium or an alkali

metal, in which each R<sup>7</sup> is independently selected from C(1-6)alkyl and phenyl;

in a β-ketoester formation reaction and thereafter, and if so needed;

10 converting P to hydrogen, and if necessary,

converting an R<sup>2A</sup>, R<sup>3A</sup> or R<sup>4A</sup> group to an R<sup>2</sup>, R<sup>3</sup> or R<sup>4</sup> group;

(c) when R<sup>1</sup> is -CH<sub>2</sub>SR<sup>6</sup> and R<sup>1</sup> and R<sup>6</sup> are as hereinbefore defined;

which comprises reacting a compound of formula (IA) or (IB);

15 in which R<sup>1</sup> is -CH<sub>2</sub>-halogen

with a compound of formula (VI):



(VI)

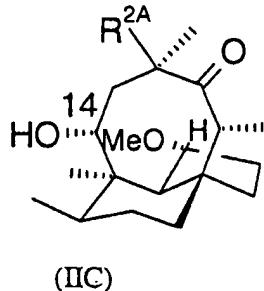
20 in which R<sup>6A</sup> is R<sup>6</sup> as defined for formula (IA) and (IB), or a group convertible to R<sup>6</sup>;

and thereafter, and if so needed;

converting R<sup>6A</sup> to R<sup>6</sup>;

(d) for a compound of formula (IA) and in which R<sup>3</sup> and R<sup>4</sup> are both hydrogen, which comprises reacting an *epi*-mutilin compound of formula (IIC):

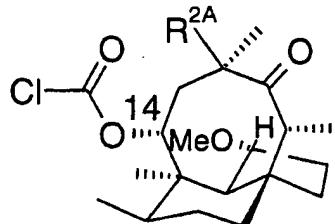
5



and where required or desired converting an R<sup>1A</sup> group to an R<sup>1</sup> group and an R<sup>2A</sup> group to an R<sup>2</sup> group; or

(e) for a compound of formula (IA) in which R<sup>3</sup> and R<sup>4</sup> are both hydrogen, which comprises reacting an *epi*-mutilin compound of formula (IVC):

15



(IVC)

wherein R<sup>2A</sup> is as hereinbefore defined;  
with a compound (V), as hereinbefore defined;  
and then treating the product with an acid;  
and where required or desired converting an R<sup>1A</sup> group to an R<sup>1</sup> group and an R<sup>2A</sup> group to an R<sup>2</sup> group.

# INTERNATIONAL SEARCH REPORT

International Application No  
PCT/EP 00/07687

A. CLASSIFICATION OF SUBJECT MATTER					
IPC 7	C07C69/738	C07C69/716	C07D213/55	C07D211/34	C07D487/08
	C07D453/02	C07D239/38	C07D285/135	C07D277/76	C07D249/14
	C07C323/52	A61K31/215	A61K31/45	A61K31/4412	A61K31/513

According to International Patent Classification (IPC) or to both national classification and IPC

## B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)  
IPC 7 C07C C07D A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BEILSTEIN Data, CHEM ABS Data

## C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	WO 99 21855 A (SMITHKLINE BEECHAM P.L.C.) 6 May 1999 (1999-05-06) cited in the application page 57 -page 62; claims -----	1

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

### \* Special categories of cited documents :

- \*A\* document defining the general state of the art which is not considered to be of particular relevance
- \*E\* earlier document but published on or after the international filing date
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- \*O\* document referring to an oral disclosure, use, exhibition or other means
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- \*8\* document member of the same patent family

Date of the actual completion of the international search

6 December 2000

Date of mailing of the international search report

18/12/2000

Name and mailing address of the ISA

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1

# INTERNATIONAL SEARCH REPORT

Interr. Application No  
PCT/EP 00/07687

**A. CLASSIFICATION OF SUBJECT MATTER**  
 IPC 7 A61K31/428 A61K31/433 A61K31/4196 A61K31/439

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**

Minimum documentation searched (classification system followed by classification symbols)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

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Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

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- \*&\* document member of the same patent family

Date of the actual completion of the international search

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Kinzinger, J

# INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/07687

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9921855 A	06-05-1999	AU 9636198 A EP 1028961 A NO 20002173 A	17-05-1999 23-08-2000 05-06-2000